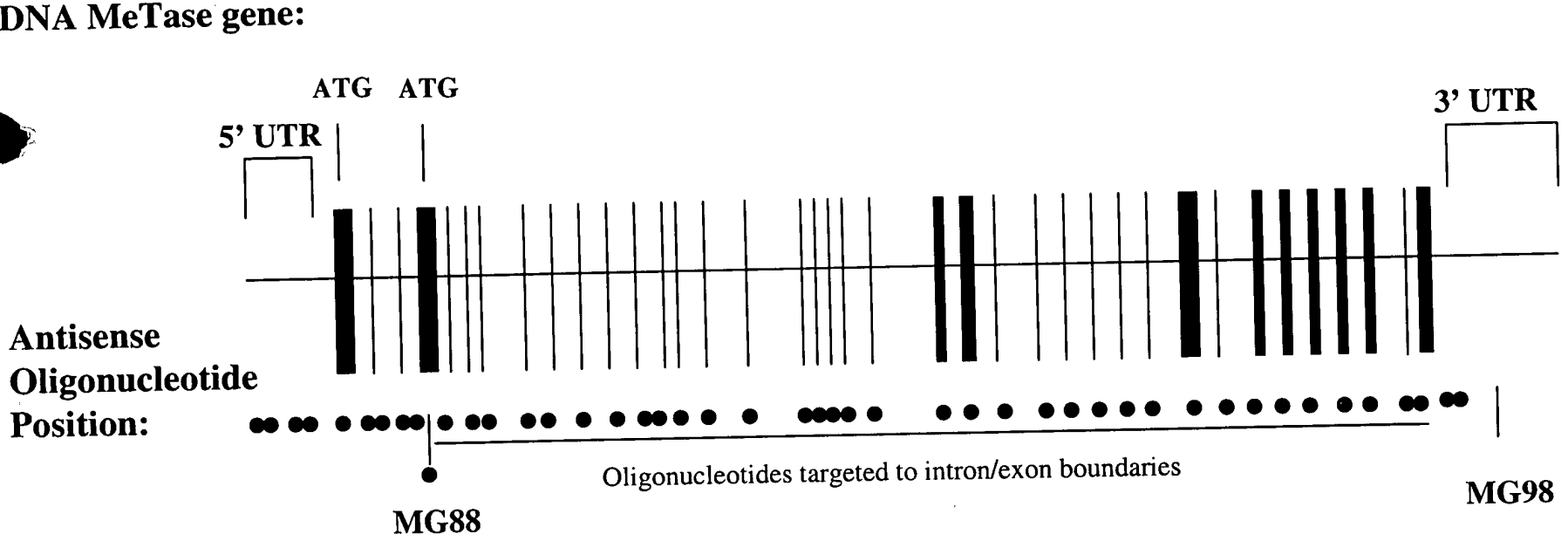

DNA MeTase gene:
ATG
ATG
5' UTR
3' UTR
Antisense
Oligonucleotide
Position:
Oligonucleotides targeted to intron/exon boundaries
MG88
MG98

FIGURE 1

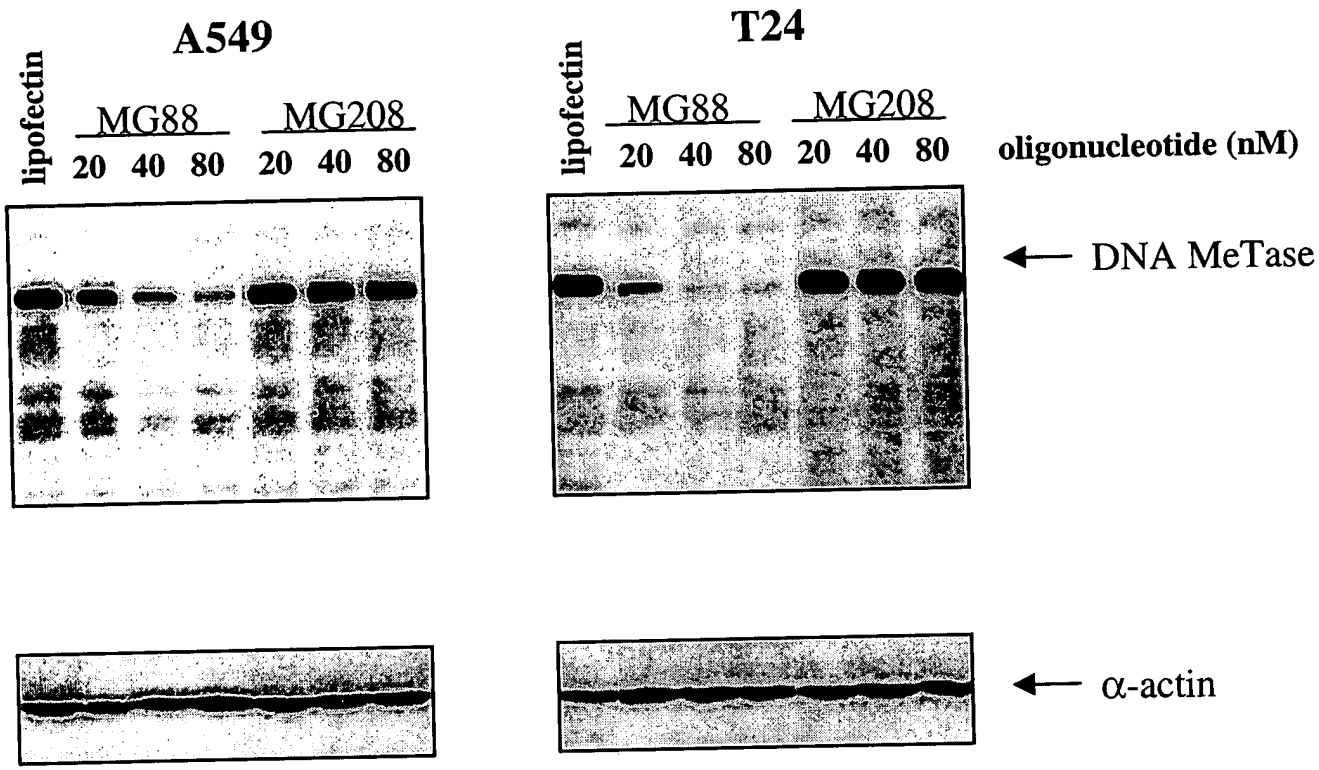

A549
T24
lipofectin
MG88
MG208
20 40 80 20 40 80
oligonucleotide (nM)
DNA MeTase
α-actin

FIGURE 2

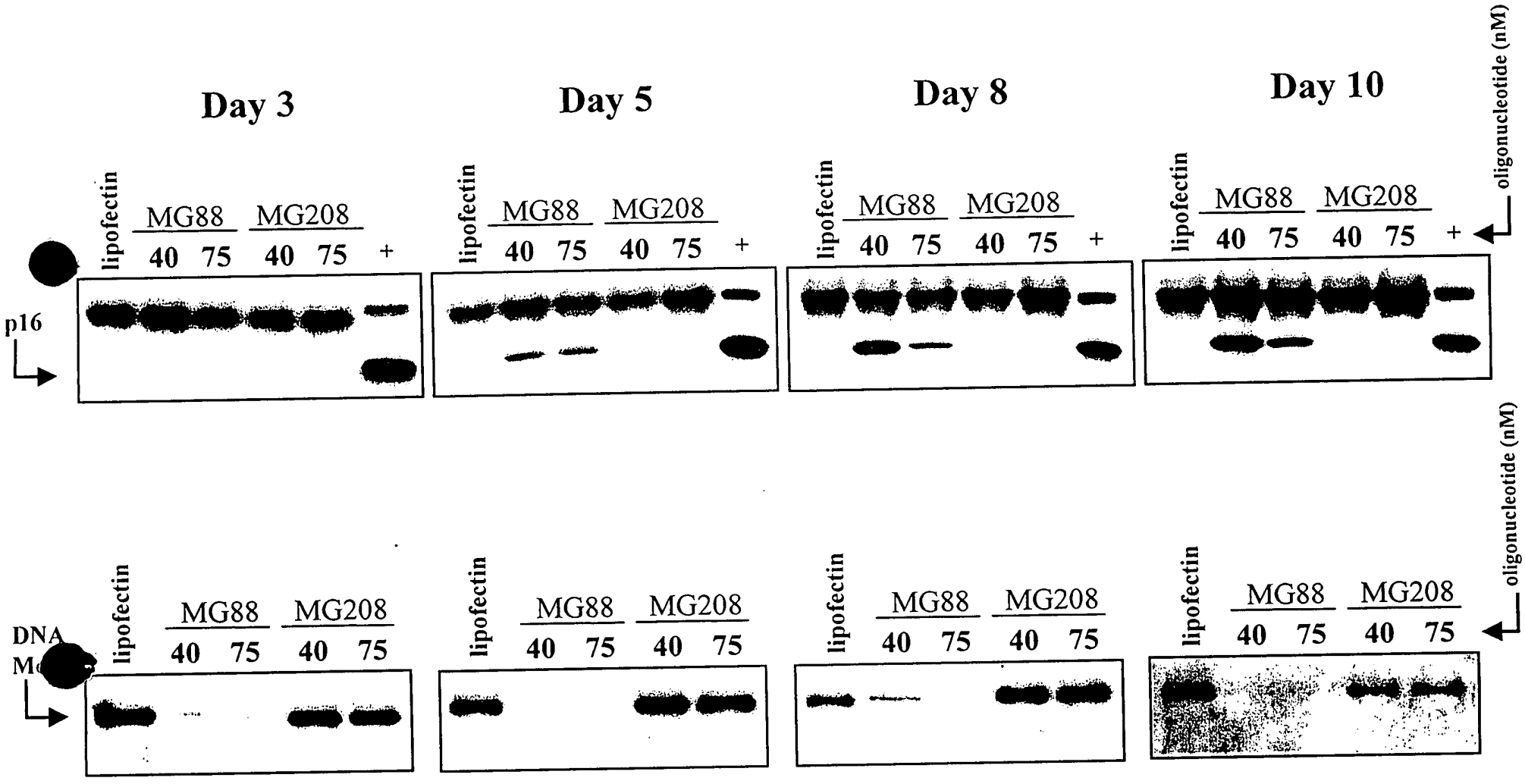

Day 3
Day 5
Day 8
Day 10
lipofectin
MG88
MG208
40 75 40 75 +
oligonucleotide (nM)
p16
DNA

FIGURE 3A

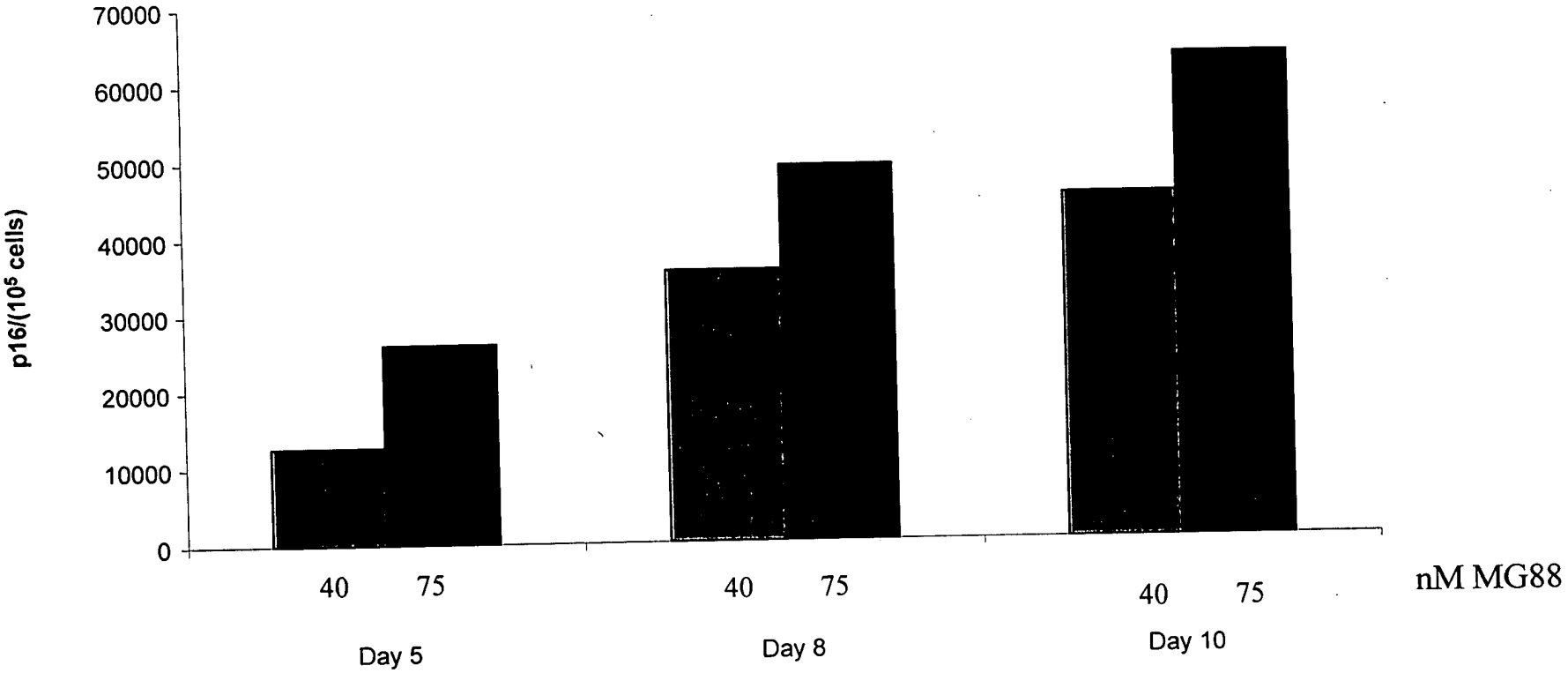

70000
60000
50000
40000
30000
20000
10000
0
p16/(10^5 cells)
40
75
40
75
40
75
nM MG88
Day 5
Day 8
Day 10

FIGURE 3B

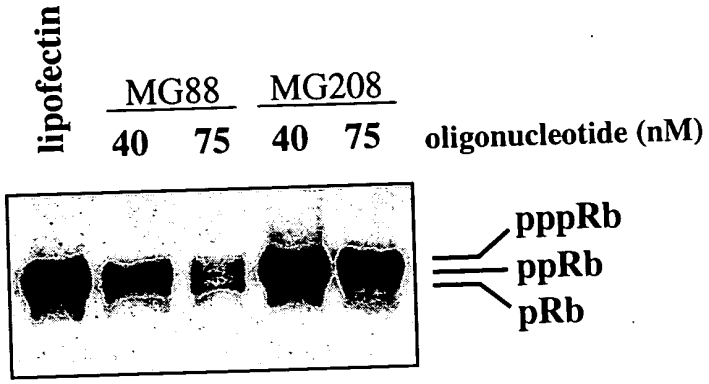

lipofectin
MG88
MG208
40
75
40
75
oligonucleotide (nM)
pppRb
ppRb
pRb

FIGURE 4

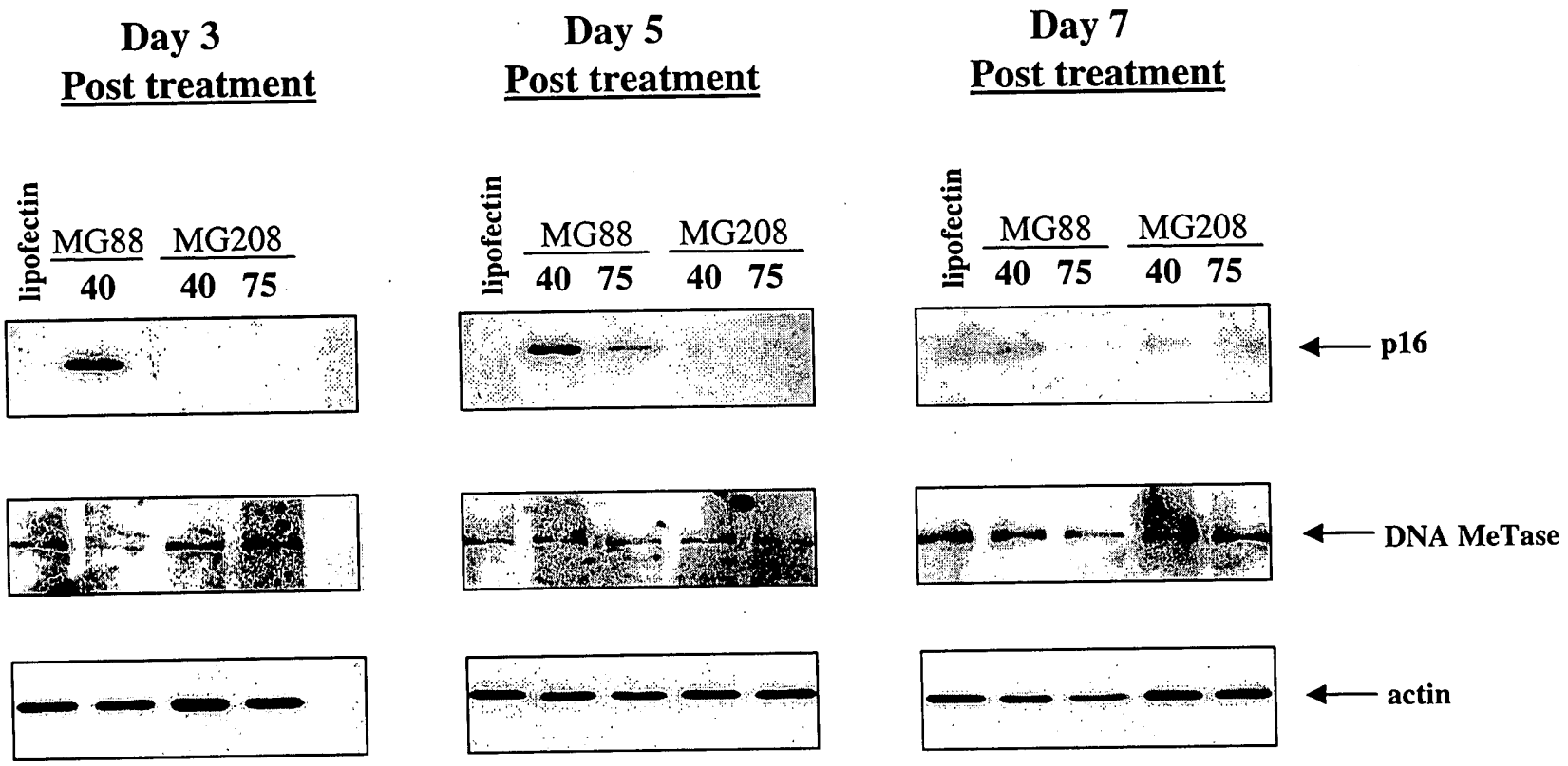

Day 3
Post treatment
Day 5
Post treatment
Day 7
Post treatment
lipofectin
MG88
MG208
40
40
75
lipofectin
MG88
MG208
40
75
40
75
lipofectin
MG88
MG208
40
75
40
75
p16
DNA MeTase
actin

FIGURE 5

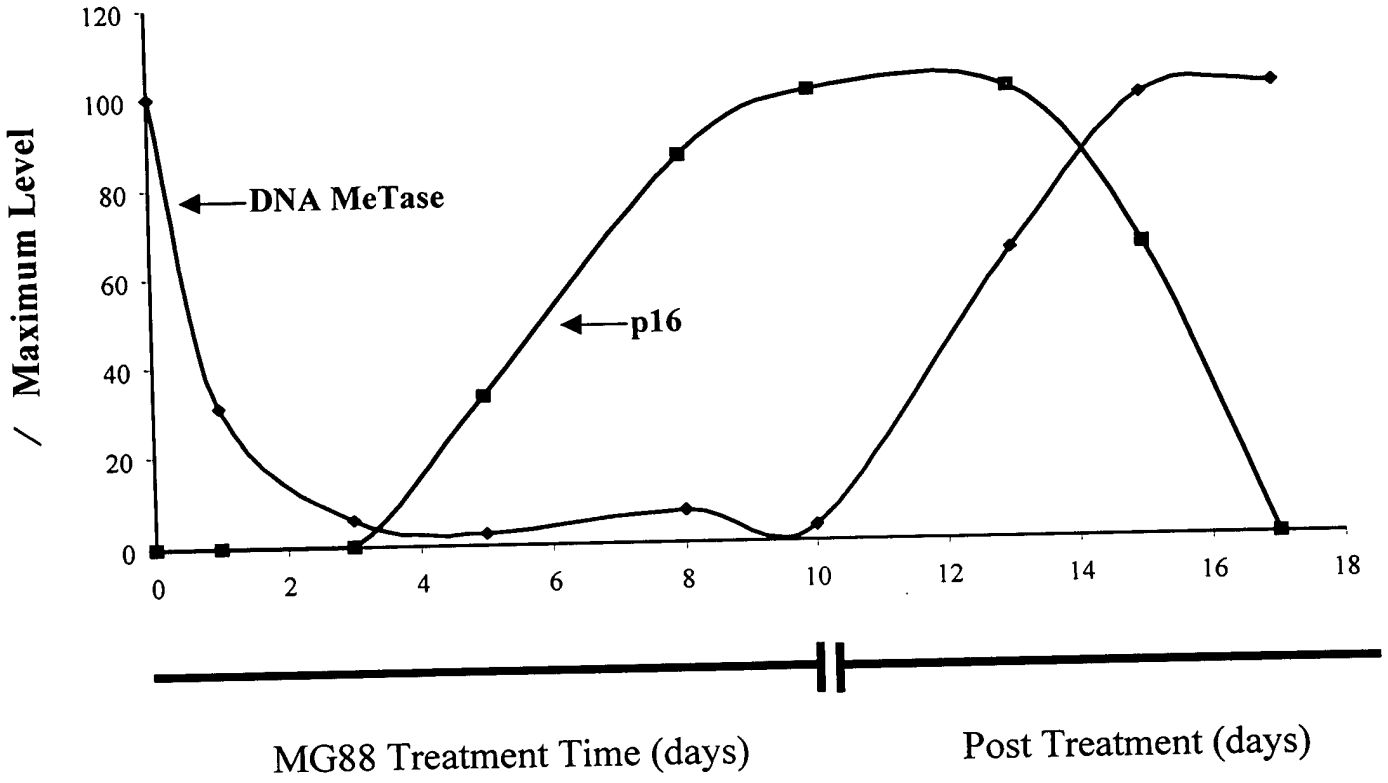

120
100
80
60
40
20
0
/ Maximum Level
DNA MeTase
p16
0
2
4
6
8
10
12
14
16
18
MG88 Treatment Time (days)
Post Treatment (days)

FIGURE 6

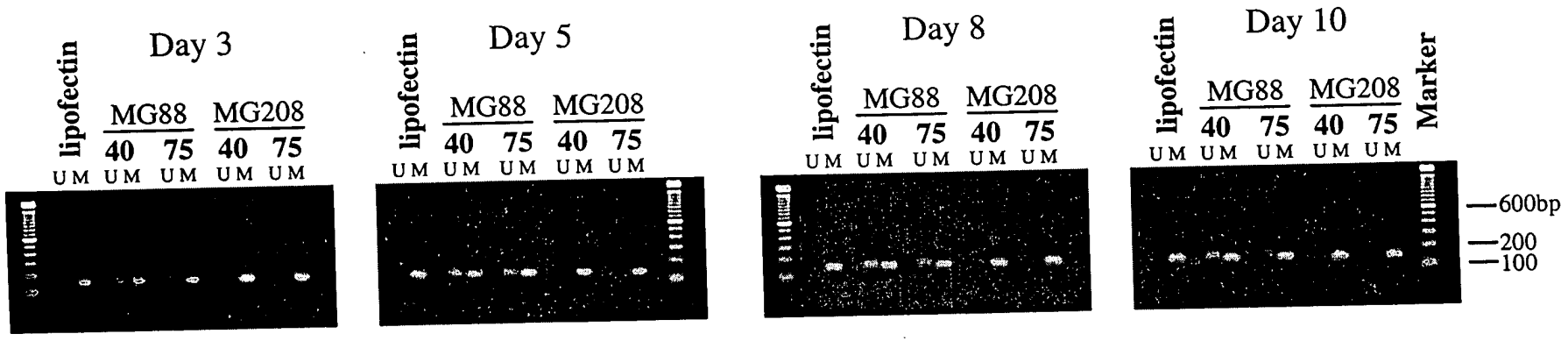
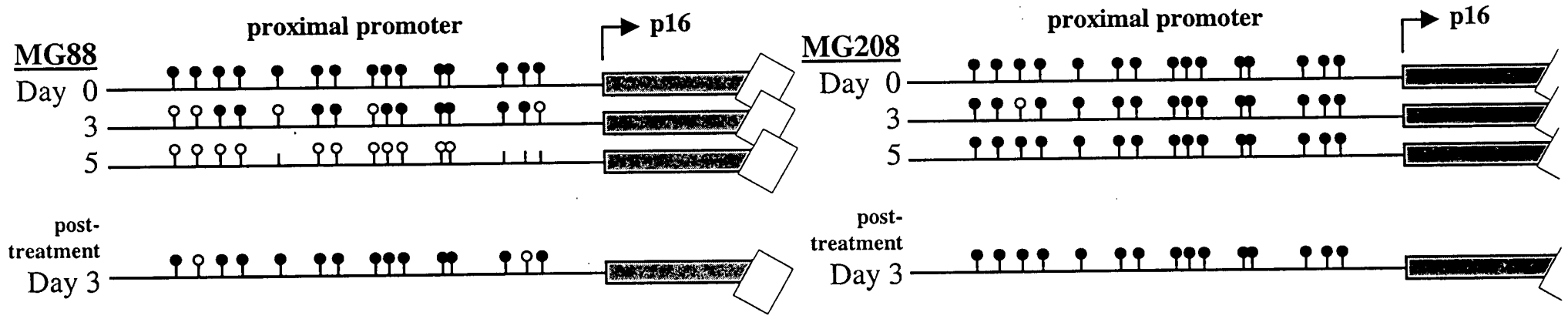

Day 3
lipofectin
MG88
MG208
40
75
40
75
UM UM UM UM UM
Day 5
lipofectin
MG88
MG208
40
75
40
75
UM UM UM UM UM
Day 8
lipofectin
MG88
MG208
40
75
40
75
UM UM UM UM UM
Day 10
lipofectin
MG88
MG208
40
75
40
75
UM UM UM UM UM
Marker
600bp
200
100

FIGURE 7



* T24 Cells

FIGURE 8

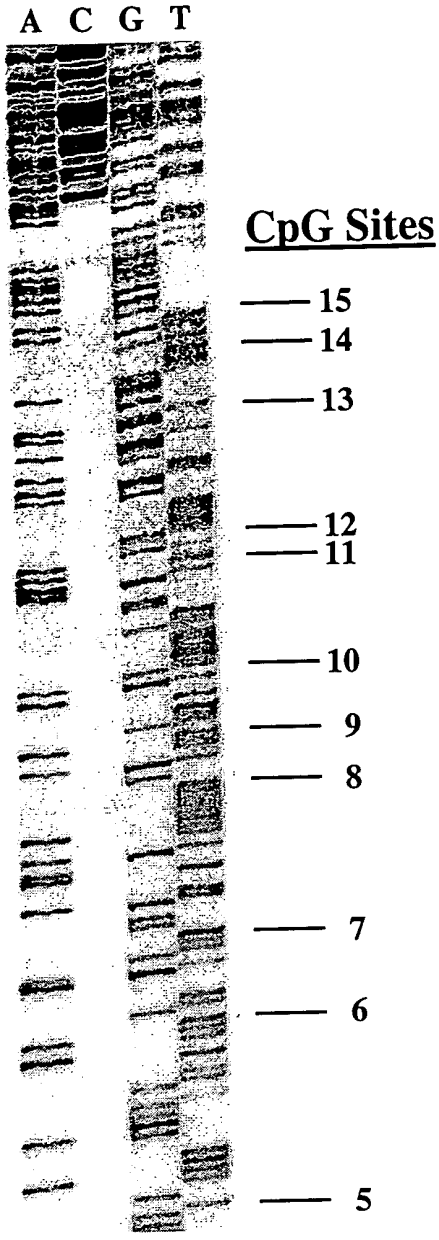


FIGURE 9A

FIGURE 9B

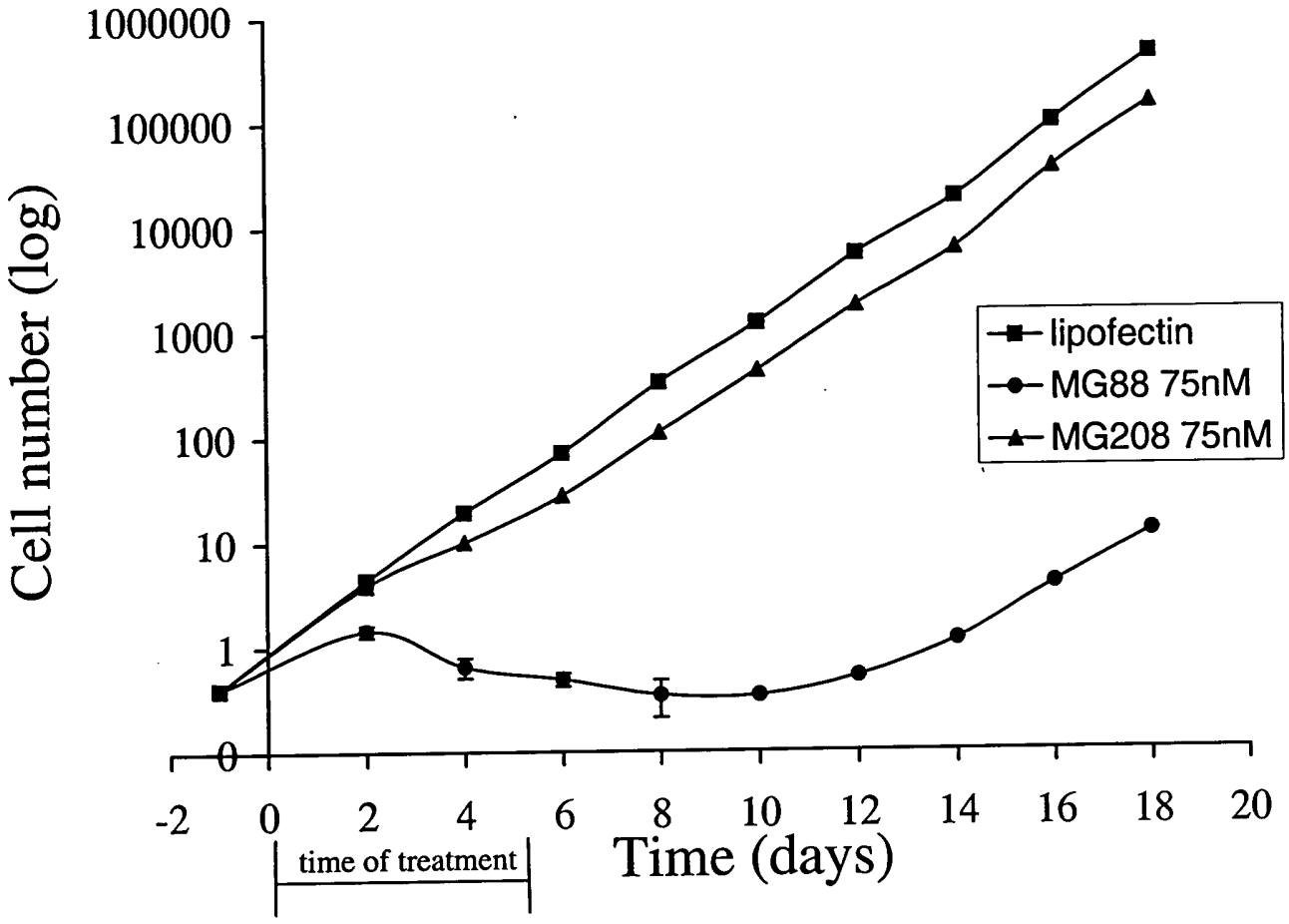
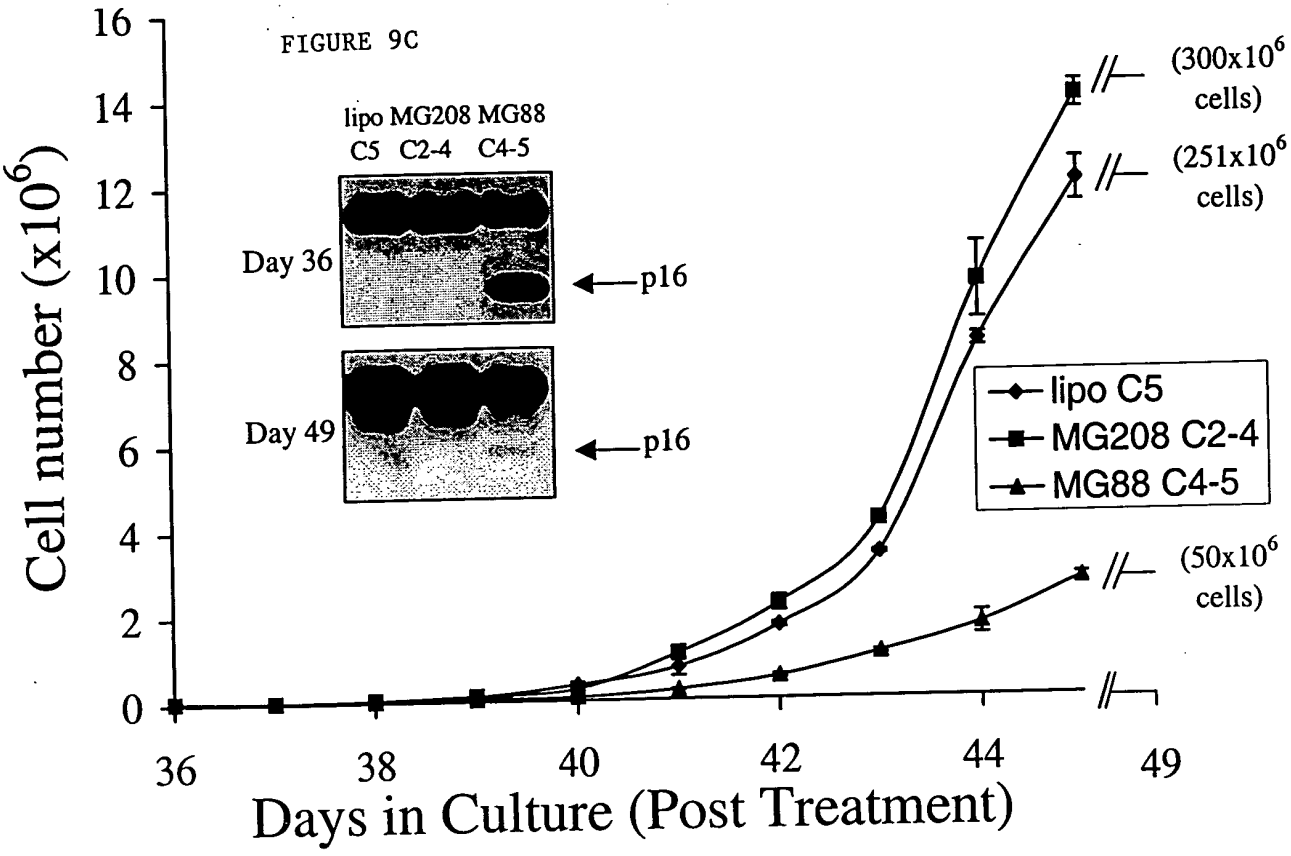

1000000
100000
10000
1000
100
10
1
0
Cell number (log)
-2
0
2
4
6
8
10
12
14
16
18
20
time of treatment
Time (days)
lipofectin
MG88 75nM
MG208 75nM

FIGURE 9C


lipo MG208 MG88
C5 C2-4 C4-5
Day 36
p16
Day 49
p16
Cell number (x10^6)
16
14
12
10
8
6
4
2
0
36
38
40
42
44
49
Days in Culture (Post Treatment)
(300x10^6 cells)
(251x10^6 cells)
(50x10^6 cells)
lipo C5
MG208 C2-4
MG88 C4-5

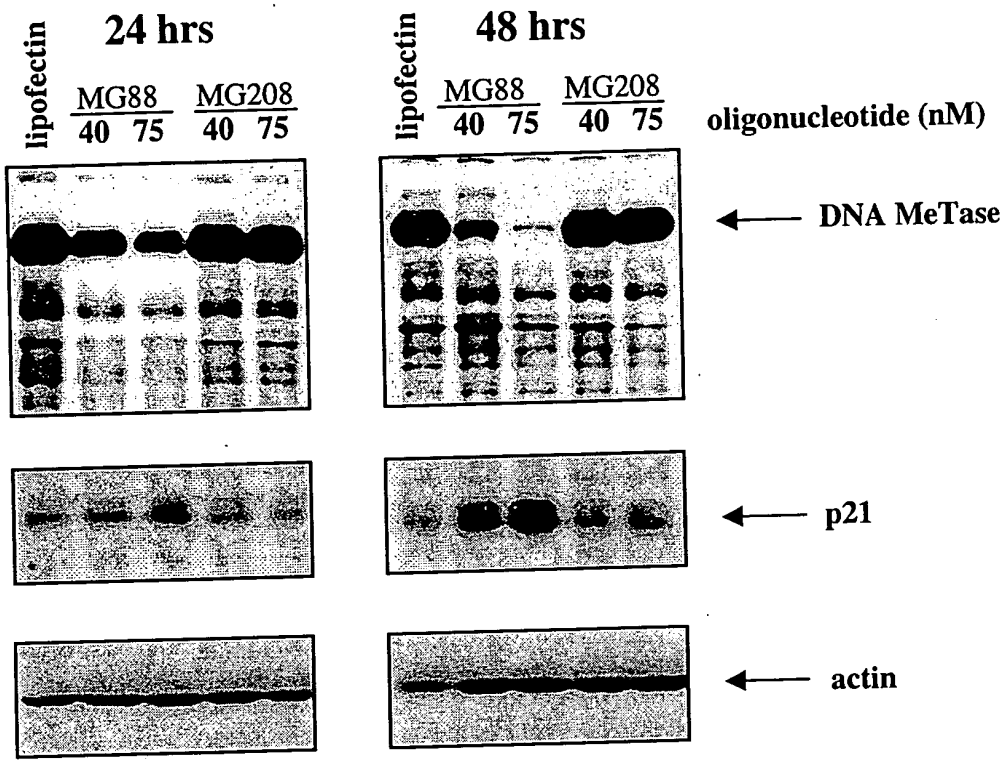

24 hrs
48 hrs
lipofectin
MG88
MG208
40 75 40 75
lipofectin
MG88
MG208
40 75 40 75
oligonucleotide (nM)
DNA MeTase
p21
actin

FIGURE 10A

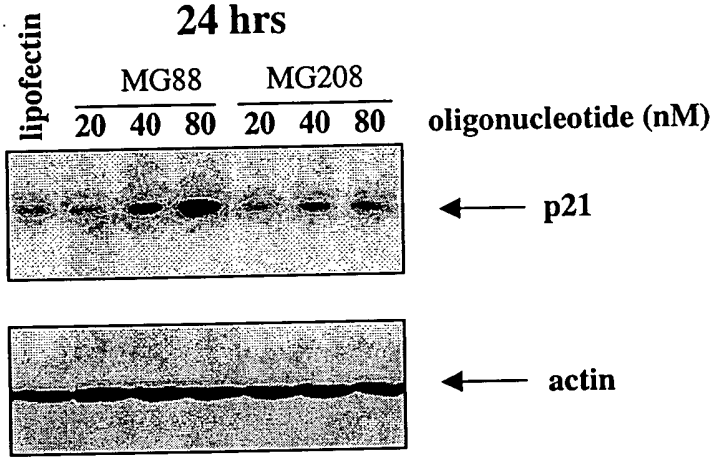

24 hrs
lipofectin
MG88
MG208
20 40 80 20 40 80
oligonucleotide (nM)
p21
actin

FIGURE 10B

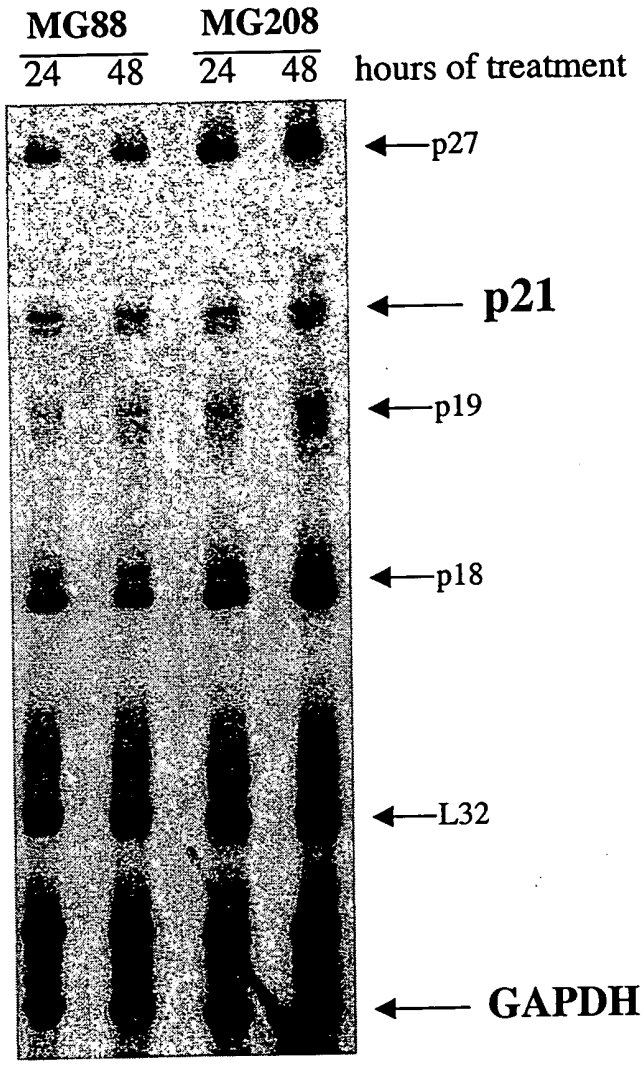
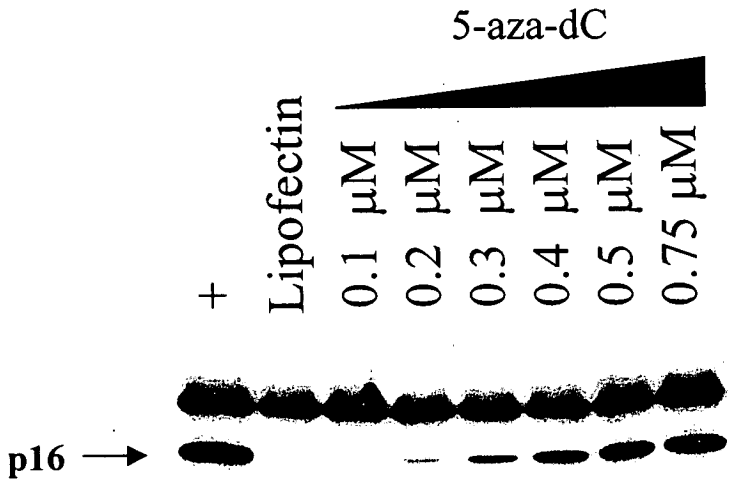

MG88
MG208
24
48
24
48
hours of treatment
p27
p21
p19
p18
L32
GAPDH

FIGURE 11

Figure 12

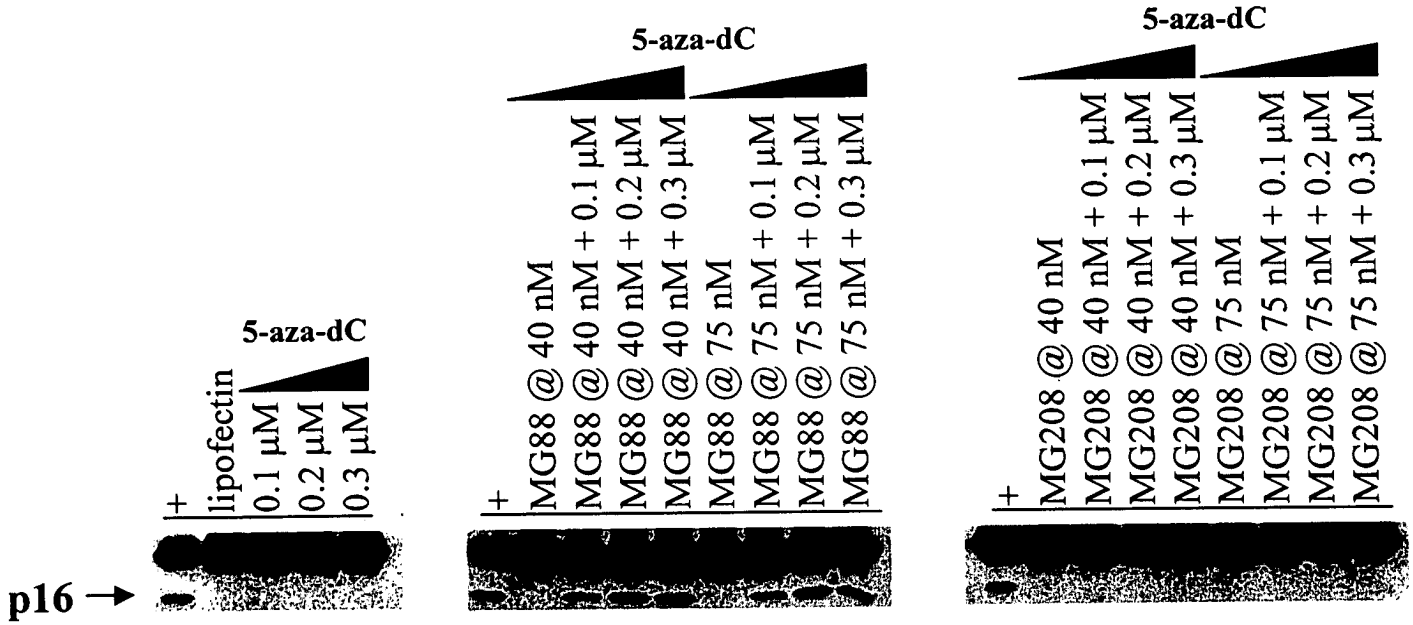
p16 reactivation in T24 cells by 5-aza-deoxycytidine treatment



T24 cells were plated and treated for three days with varying concentrations of 5aza-dC. The p16 protein was immunoprecipitated from celllysates and a Western analysis was performed.

Figure 13

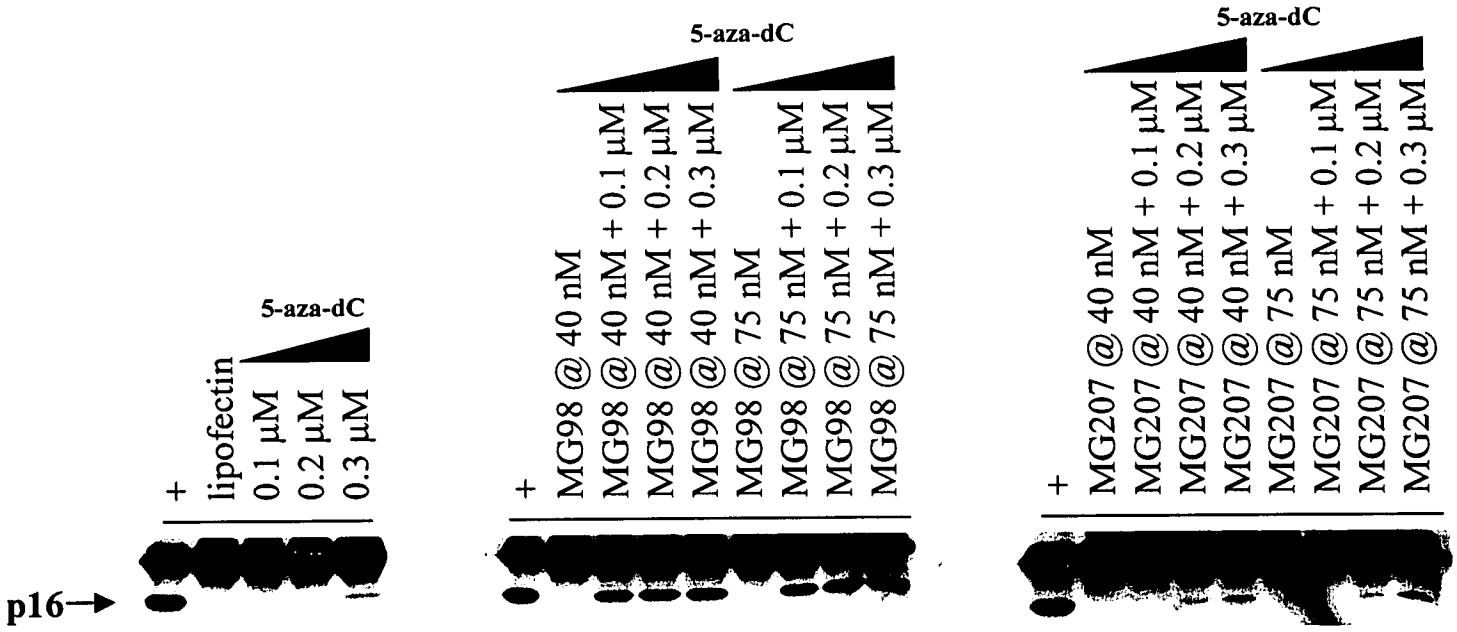
Synergistic reactivation of p16 in T24 cells by treatment with antisense to DNA methyltransferase (MG88) and 5-aza-deoxycytidine.



T24 cells were plated and transfected with either MG88 or MG208 and treated with varying concentrations of 5-aza-dC every day for three days. The p16 protein was immunoprecipitated from cell lysates and a Western analysis was performed.

Figure 14

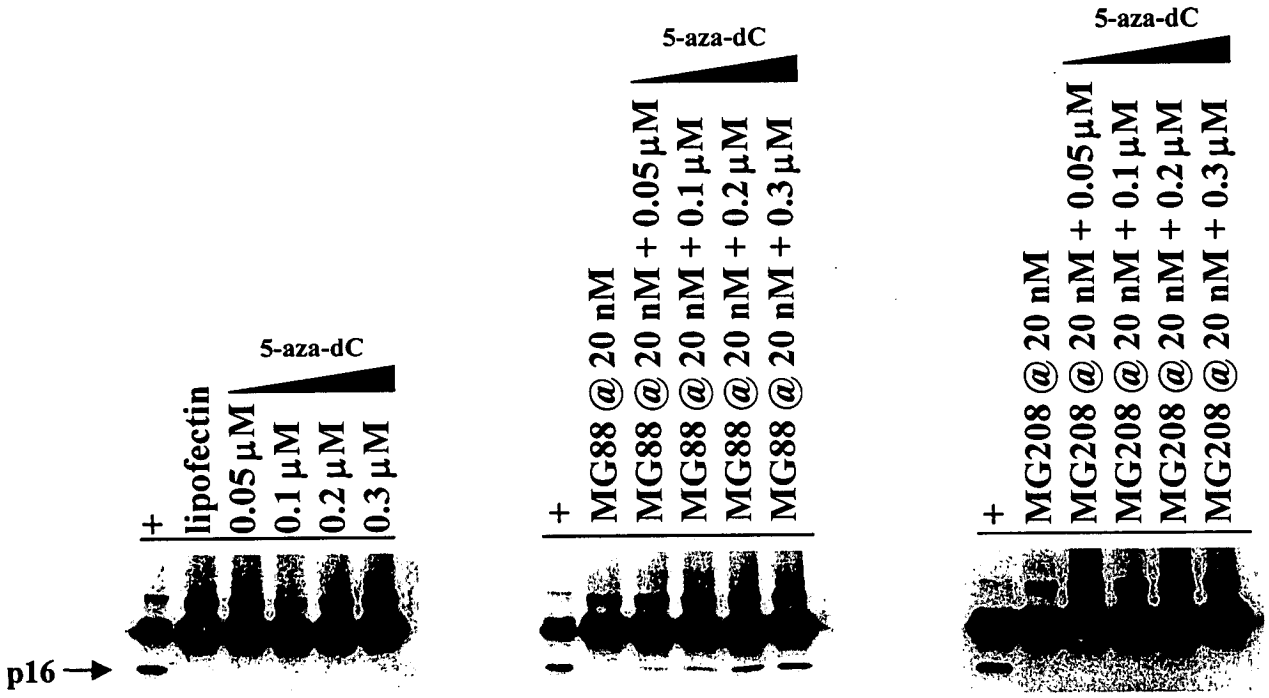
Synergistic reactivation of p16 in T24 cells by treatment with antisense to DNA methyltransferase (MG98)and 5-aza-deoxycytidine.



T24 cells were plated and transfected with either MG98 or MG207 and treated with varying concentrations of 5-aza-dC every day for three days. The p16 protein was immunoprecipitated from cell lysates and a Western analysis was performed.

Figure 15

Synergistic reactivation of p16 in T24 cells by treatment with low dose antisense to DNA methyltransferase (MG88) and 5-aza-deoxycytidine.



T24 cells were plated and transfected with either MG88 or MG 208 and treated with varying concentrations of 5-aza-dC every day for three days. The p16 protein was immunoprecipitated from cell lysates and a Western analysis was performed.

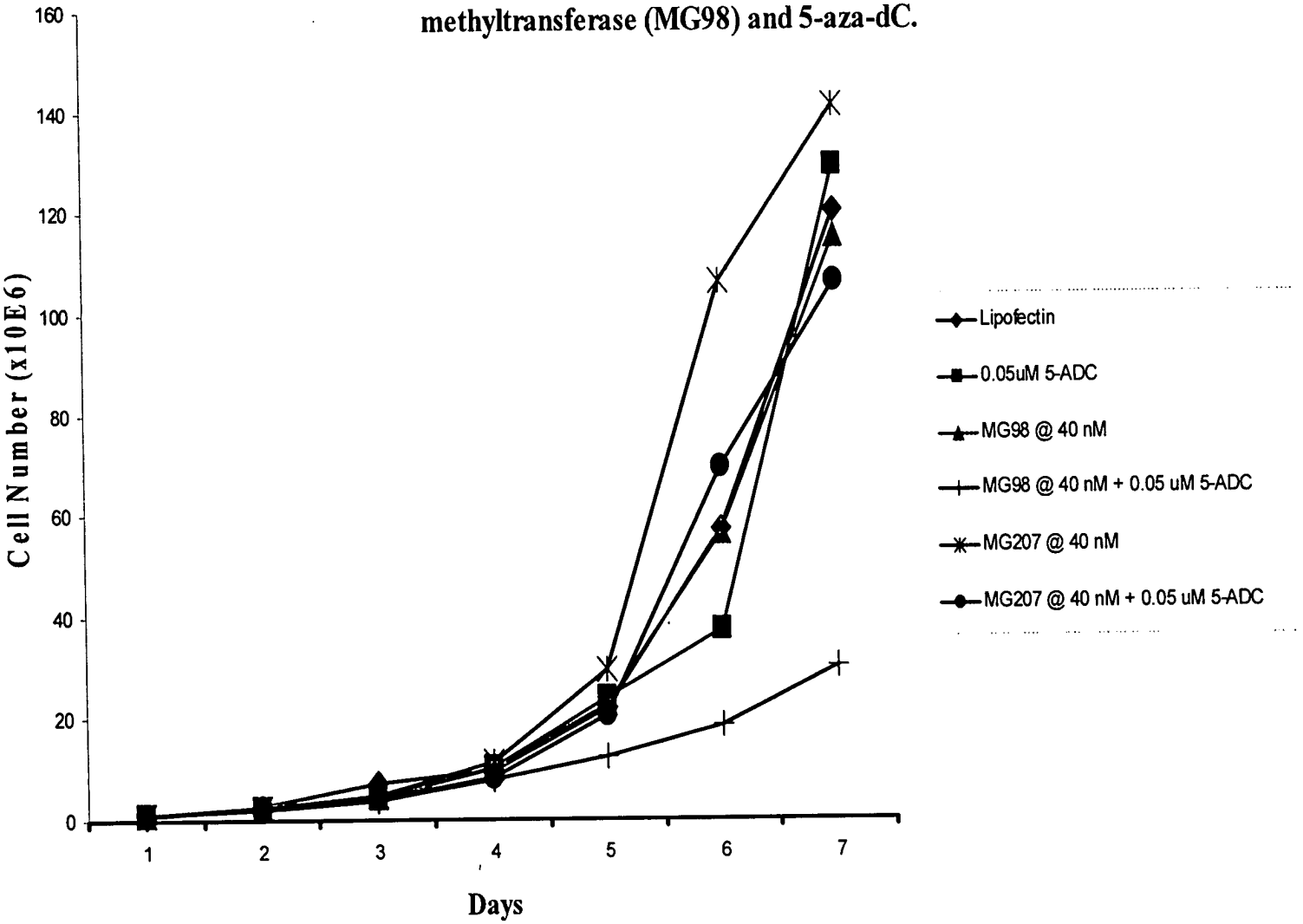


FIGURE 16

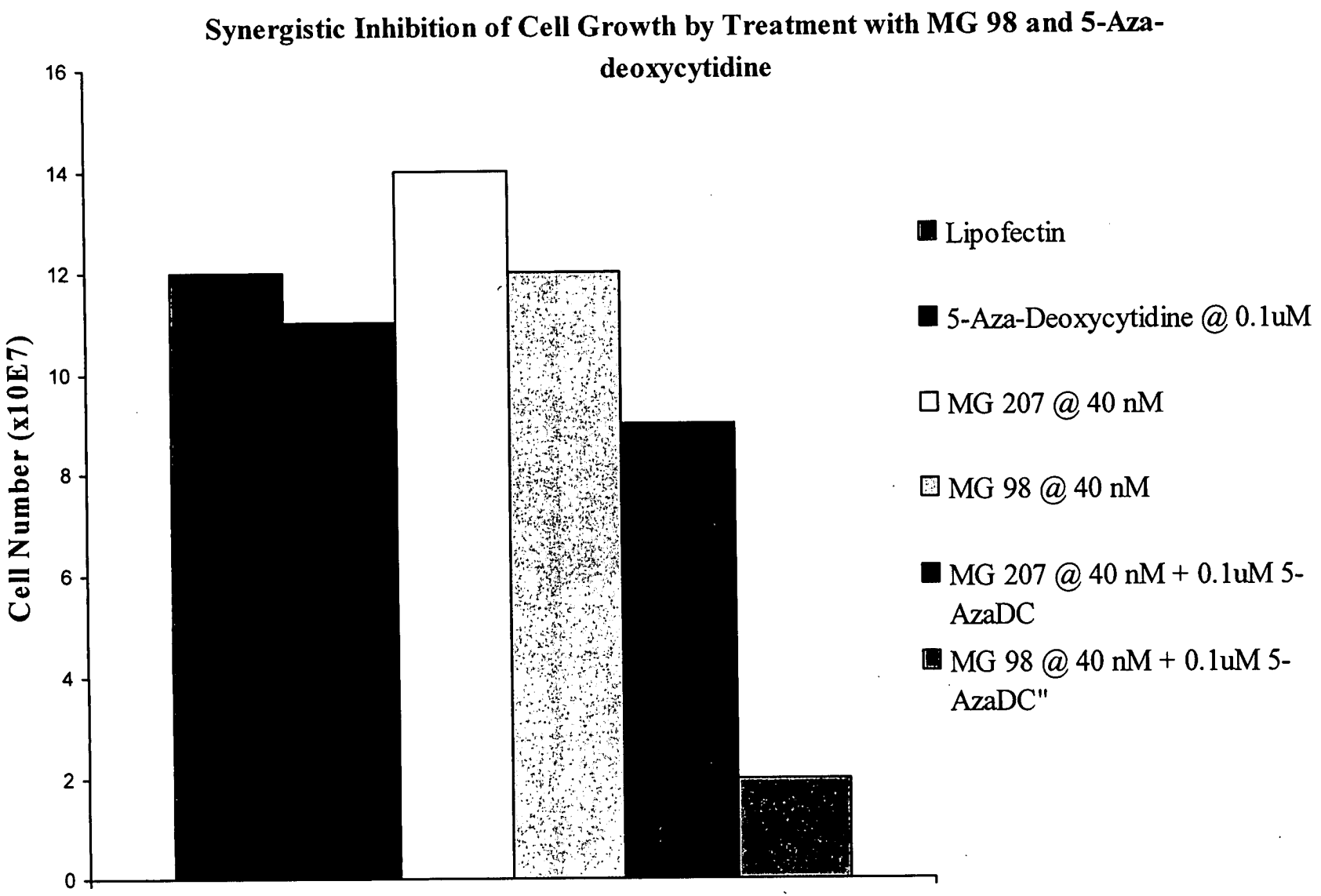

Synergistic Inhibition of Cell Growth by Treatment with MG 98 and 5-Aza-deoxycytidine
Cell Number (x10E7)
16
14
12
10
8
6
4
2
0
Lipofectin
5-Aza-Deoxycytidine @ 0.1uM
MG 207 @ 40 nM
MG 98 @ 40 nM
MG 207 @ 40 nM + 0.1uM 5-AzaDC
MG 98 @ 40 nM + 0.1uM 5-AzaDC"

FIGURE 17

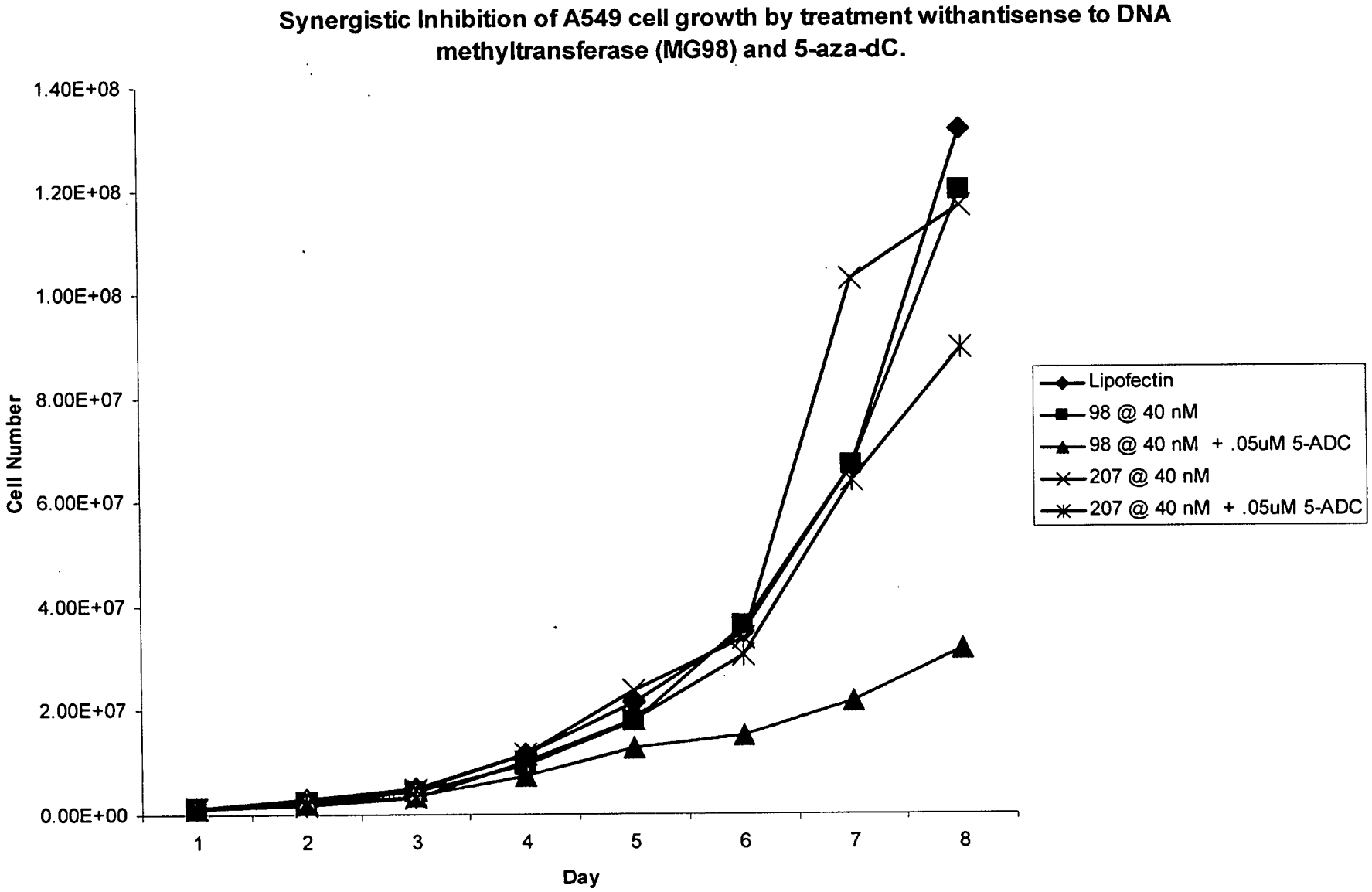

Synergistic Inhibition of A549 cell growth by treatment withantisense to DNA methyltransferase (MG98) and 5-aza-dC.
1.40E+08
1.20E+08
1.00E+08
8.00E+07
6.00E+07
4.00E+07
2.00E+07
0.00E+00
Cell Number
1
2
3
4
5
6
7
8
Day
Lipofectin
98 @ 40 nM
98 @ 40 nM + .05uM 5-ADC
207 @ 40 nM
207 @ 40 nM + .05uM 5-ADC

FIGURE 18

In vivo Synergistic Antitumor Activity of Antisense to Human DNA Methyltransferase (MG98) Combined with a Small Molecule in Human Colon Cancer Model Colo 205.

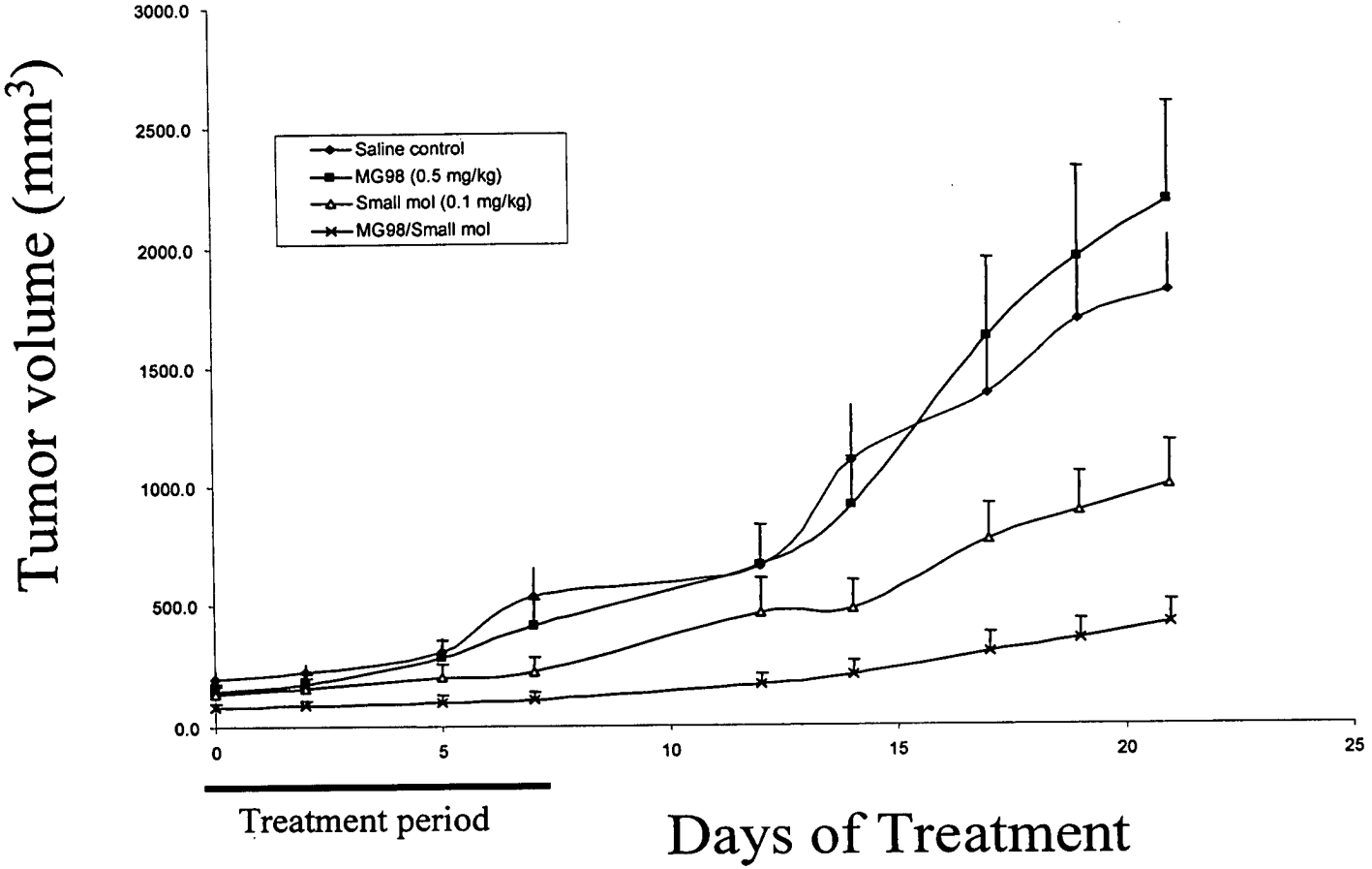


FIGURE 19

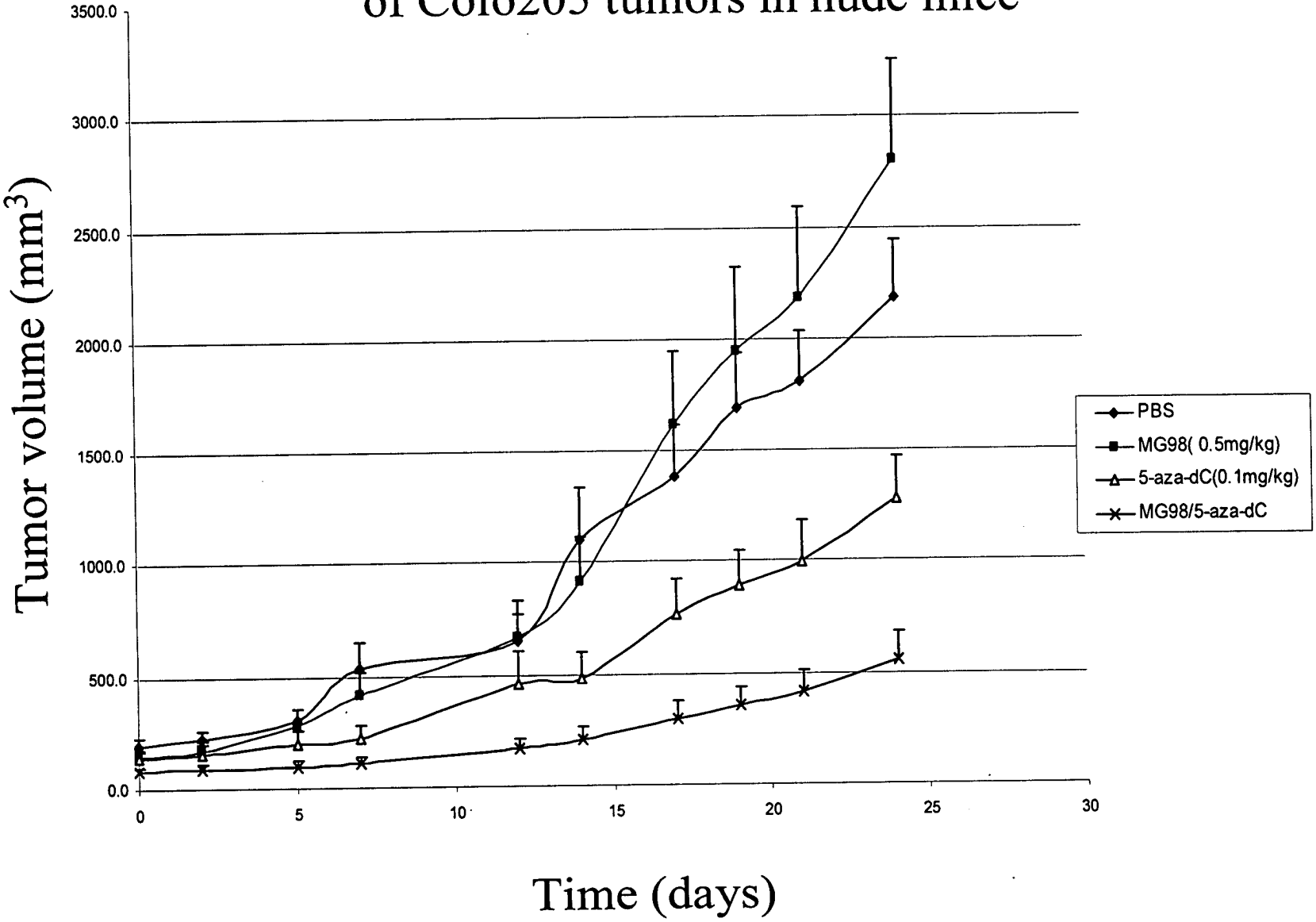

Combination of MG98 and 5-aza-deoxycytosine on growth of Colo205 tumors in nude mice
Tumor volume (mm3)
3500.0
3000.0
2500.0
2000.0
1500.0
1000.0
500.0
0.0
0
5
10
15
20
25
30
Time (days)
PBS
MG98(0.5mg/kg)
5-aza-dC(0.1mg/kg)
MG98/5-aza-dC

FIGURE 20A

In vivo Synergistic Antitumor Activity of Antisense to Human DNA Methyltransferase (MG98) Combined with 5-aza-2-deoxycytidine in Human Colon Cancer Model Colo 205.

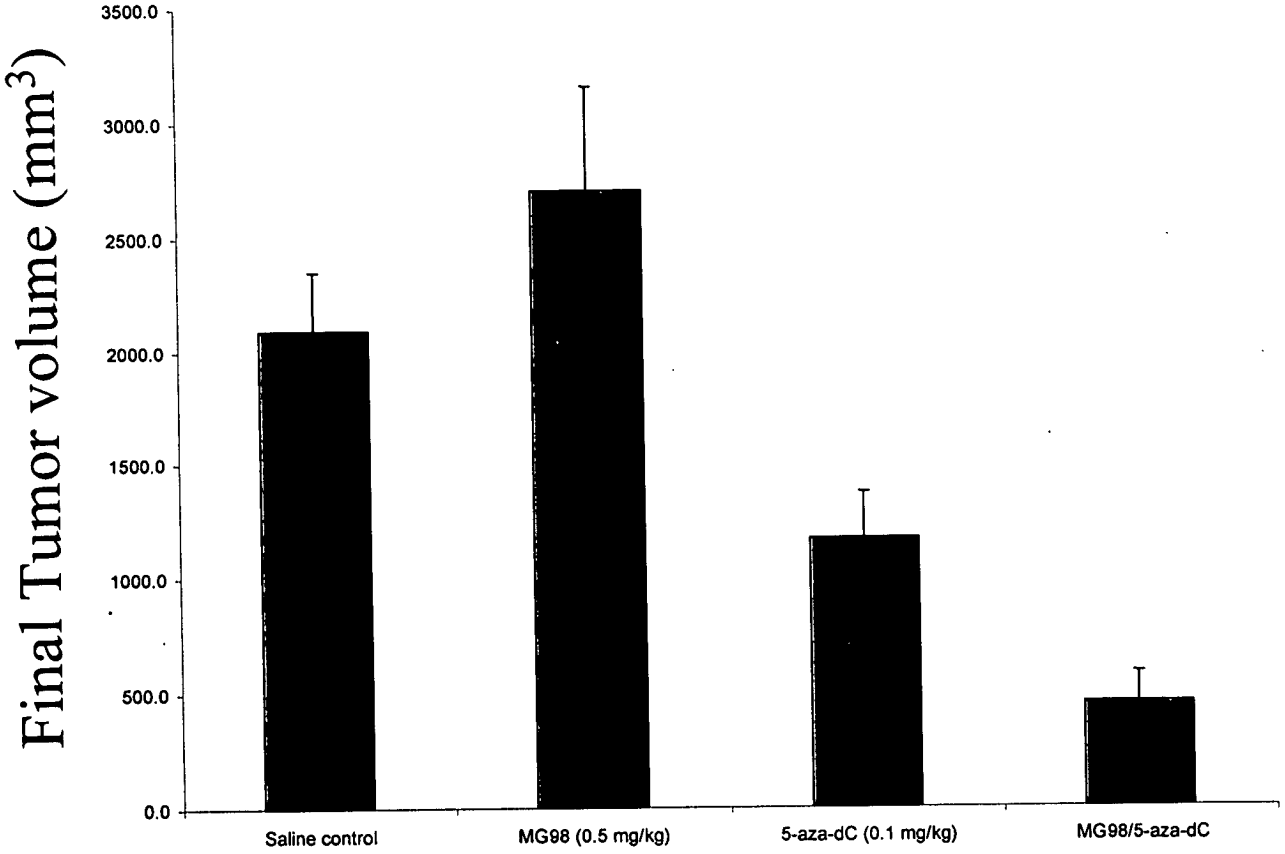
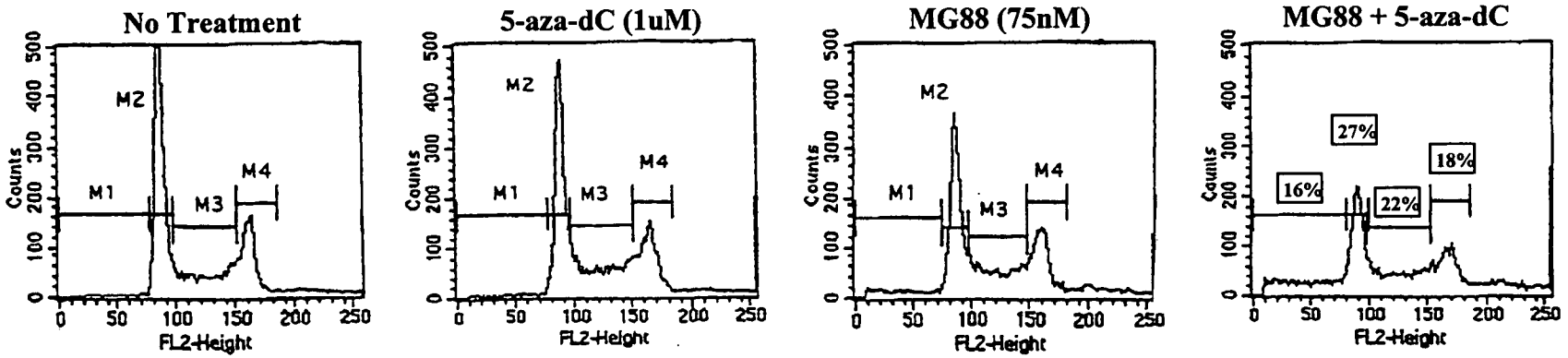


Fig. 7 . **Antitumor activity of combination of MG98 and 5-aza-2-deoxycytidine.** Groups are: Saline control, MG98 (0.5 mg/kg/day), 5-aza-2-deoxycytidine (0.1 mg/kg/day), MG98 (0.5 mg/kg/day) and 5-aza-2-deoxycytidine (0.1 mg/kg/day). Groups consisted of six animals each. Error bars represent SEM. Group MG98/5-aza-dC was statistically different ($p<0.05$) from bothsaline treated group and from 5-aza-dC treated group. Group MG98 was not significantly different than saline control group.

FIGURE 20B

Schedule Independent Inhibition of Cell Cycle Progression by Combination of DNA MeTase Antisense inhibitor (MG88) and DNA MeTase Small Molecule Inhibitor (5-aza-dC).

Schedule A: DNA MeTase Antisense Inhibitor (MG88) followed by Small Molecule Inhibitor (5-aza-dC)



Schedule B: Small Molecule Inhibitor (5-aza-dC) followed by DNA MeTase Antisense Inhibitor (MG88)

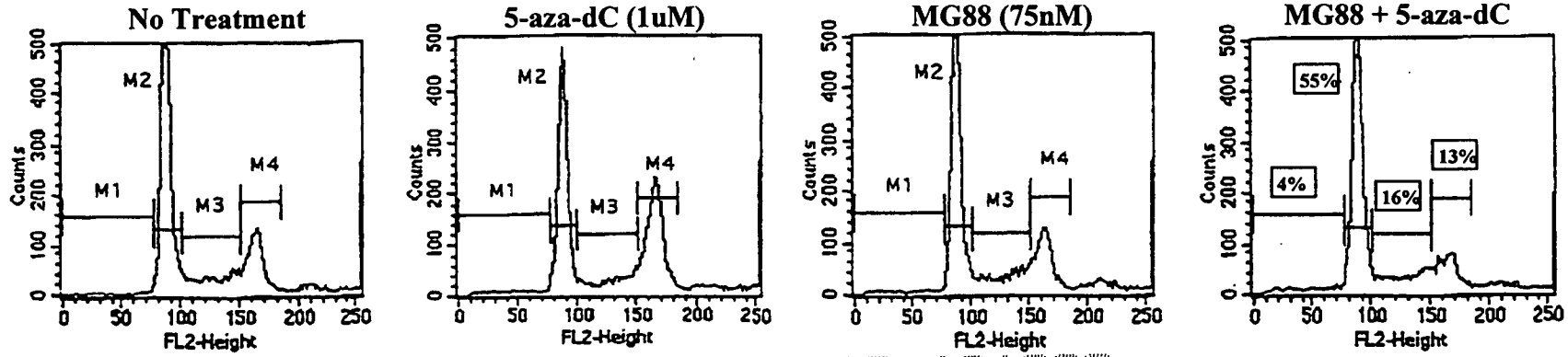


FIGURE 21

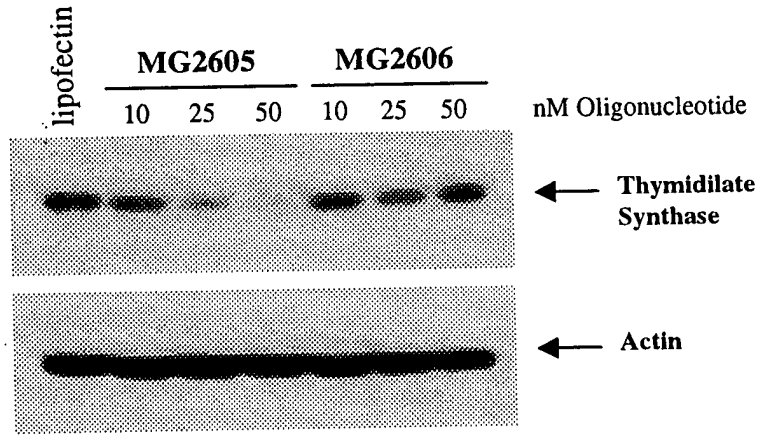
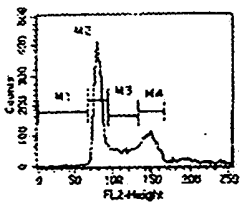
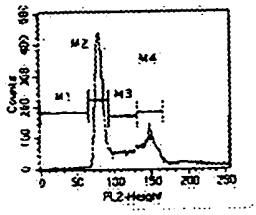

lipofectin
MG2605
MG2606
10
25
50
10
25
50
nM Oligonucleotide
Thymidilate Synthase
Actin

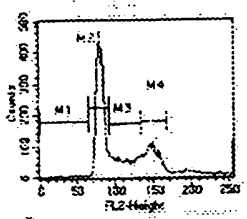
Figure 22



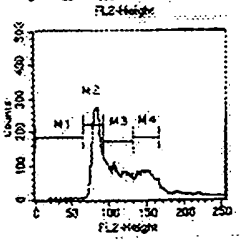
Lipofetcin



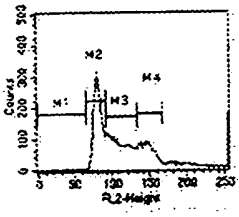
Mismatch Control



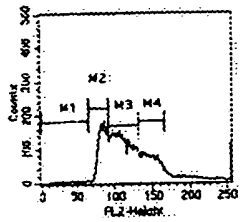
TS Antisense (25nM)



5-FU (500nM)



5-FU (500nM) +
Mismatch (25nM)



5-FU (500nM) +
TS Antisense (25nM)

Figure 23

Cell cycle analysis of cells treated with TS antisense oligo (25nM) and 5-FU (5uM)

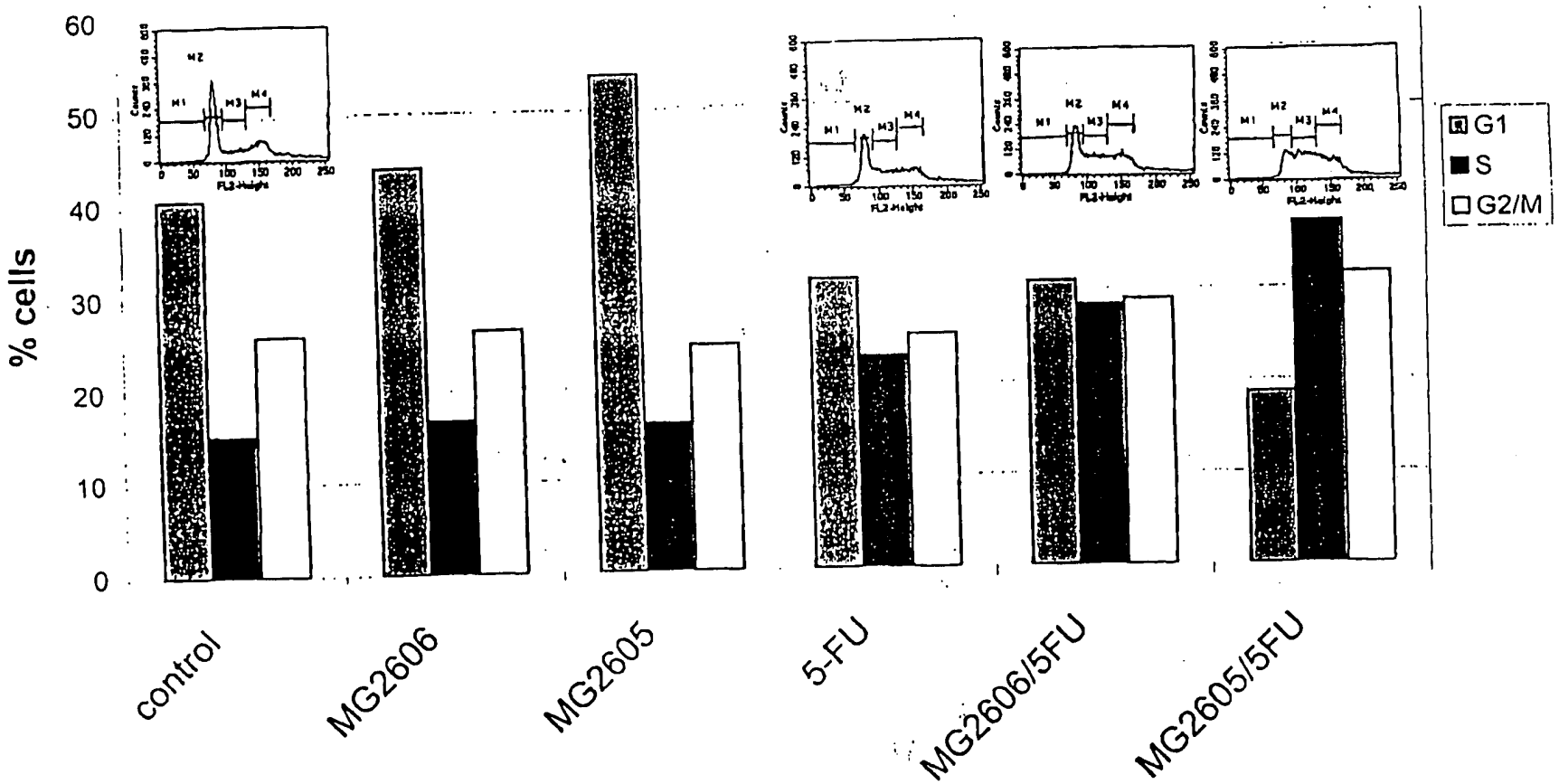


FIGURE 24A

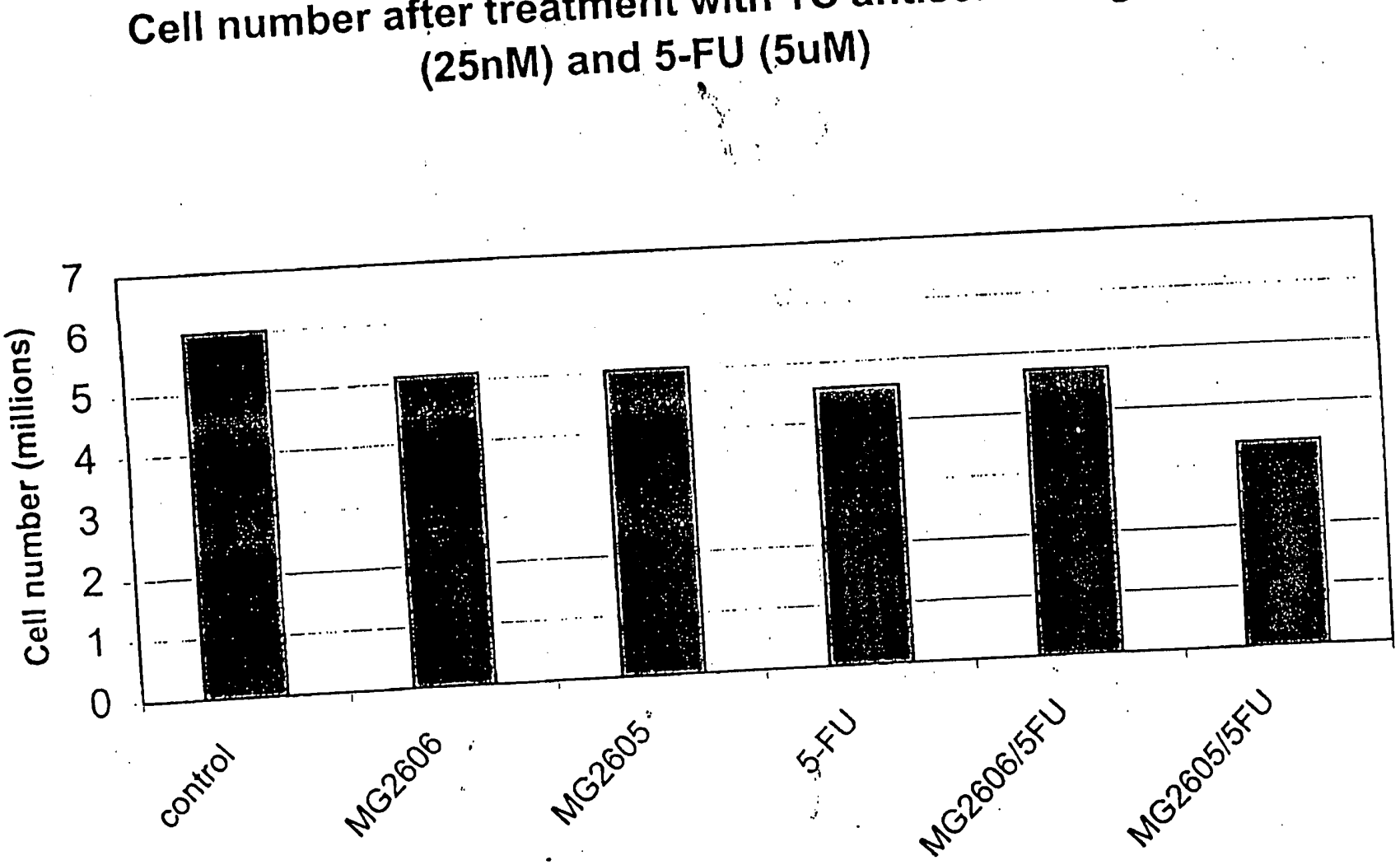

Cell number after treatment with TS antisense oligo (25nM) and 5-FU (5uM)
Cell number (millions)
0
1
2
3
4
5
6
7
control
MG2606
MG2605
5-FU
MG2606/5FU
MG2605/5FU

FIGURE 24B

Synergistic Induction of p21WAF1/CIP by Combination of HDAC Antisense and TSA

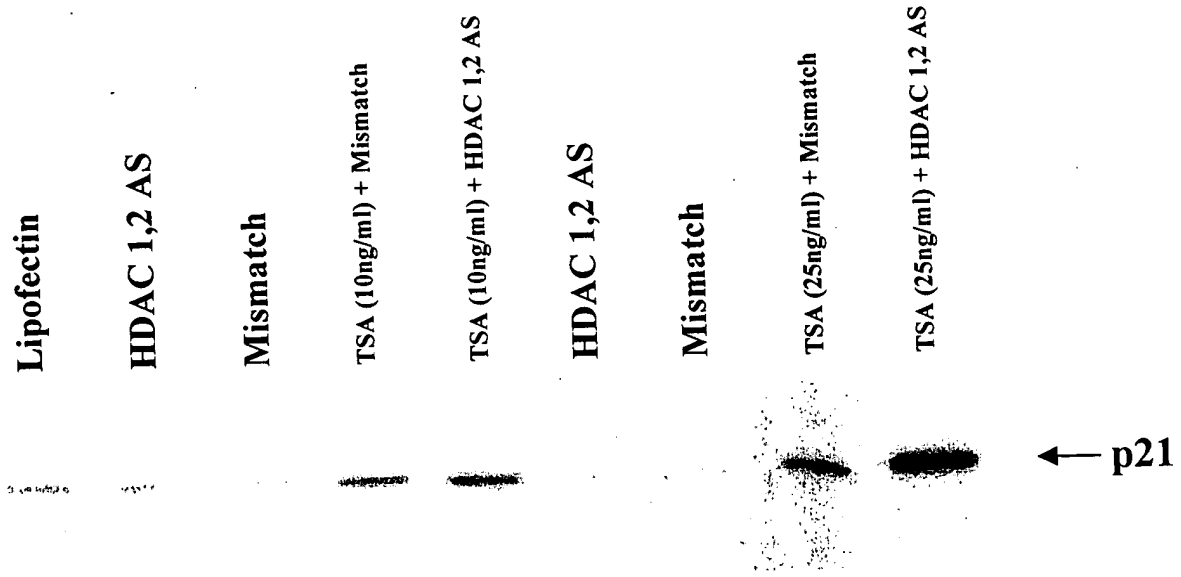


FIGURE 25